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A CONIDIAL GERMINATION TEST IN ANTIFUNGAL EVALUATIONS USING DERMATOPHYTIC ORGANISMS*

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ABSTRACT

The possiblity of using a conidial germination test in the evaluation of antifungal chemicals was explored using phenol and *T. mentagrophytes*. The results suggest the feasibility of such a method under carefully controlled conditions. Preventing clumping of conidia and germ tubes will require further work.

There has been a wide variety of test methods used in evaluating fungicidal substances *in vitro* against dermatophytic organisms. It is not the purpose here to review these methods but they have varied from conidial involvement to the use of agar plates and isolated agar discs. It might be useful to have a spore germination test method similar to that used by the American Phytopathological Society for plant pathogens (1, 2). Such a possibility was briefly explored from the point of view of germination using phenol as the antifungal agent.

MATERIALS AND METHODS

A slide technique such as used for plant fungi is not too practical for the conidia of dermatophytic organisms due to pathogenicity and slow germination. The tube germination test might be just as useful and would involve the mixing of a conidial suspension of the organism with a dilution of the fungicide in a dilute nutrient medium.

Preparation of conidial suspension. Cultures of *T. mentagrophytes* 382 were grown on Sabouraud's agar medium in 500 ml Erlenmeyer flasks for 15 days under conditions of normal daylight and darkness and at a temperature of 27 ± 2 C. Twenty-five ml of sterile saline was poured into each of 5 flasks and the surfaces of the cultures lightly brushed with a sterile test tube brush followed by gentle shaking for several minutes. The crude suspensions were combined and filtered through a sterile 100 ml Seitz filter containing washed Whatman 43 filter paper to remove mycelial fragments.

The filtered suspension was poured into a previously sterilized Millipore Hydrosol filter holder with an HA (0.45 u) filter disc. The conidia were washed with 50 ml portions of sterile saline (1000 ml) without permitting the liquid on the filter bed to go below one-half inch in depth. The last 50 ml of saline and washings was transferred to a calibrated flask. The conidia were counted by hemocytometer and the suspension adjusted to contain 2,000,000 conidia per ml.

Growth medium. The evalution of a large number of inorganic additions to a medium of peptone and glucose was made and the following medium provided between 90 and 100% germination in approximately 24 hours: Peptone (Bacto-Peptone, Difco) 0.2%, glucose 0.1%, sodium nitrate 0.2%, potassium dihydrogen phosphate 0.1%, magnesium sulfate, $7H_2O$ 0.1%, potassium chloride 0.05%, ferrous sulfate, $7H_2O$ 0.0001%, and double-distilled water to make 100%.

Test procedure. One ml of conidial suspension was added to a suitable dilution of the test compound in a sterile test tube and the tubes shaken in a water-bath shaker together with suitable control tubes at 27 ± 2 C for the required lengths of time. Periodic counts were made by removing a sample to a hemocytometer chamber and counting under high power both those conidia that had germinated and the total number of conidia. Germination was considered to be the appearance of parallel sides in the germ tube. Since all conidia do not germinate even under optimum conditions the following equation was used to compensate for the lack of germination of conidia:

$$\frac{\text{Number of conidia germinated in the trial}}{\text{Per cent germination of control}} \times \text{total count of trial} \times 10^4 = \text{Corrected } \% \text{ germination}$$

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TABLE

Corrected per cent inhibition of germination of conidia of T. mentagrophytes *from two trials made in the presence of varying concentrations of phenol*

Trial 1				Trial 2			
Phenol conc.	Number of conidia counted	Number of *conidia* germinated	Corrected* % *inhibition* of germination	Phenol conc.	Number of conidia counted	Number of conidia germinated	Corrected* % inhibition of germination
Control	110	100	0	Control	340	290	0
0.05	96	56	35.8	0.1	351	219	26.6
0.10	116	62	40.8	0.2	327	178	36.0
0.15	130	66	44.1	0.4	330	111	60.5
0.25	100	46	49.3	0.6	369	36	88.6
0.30	138	58	53.6	0.8	313	14	94.7
0.40	122	40	63.9	0.9	313	5	98.1
0.50	122	24	78.3	1.0	325	0	100.0

* Control germination was 91% in Trial 1 and 85.2% in Trial 2.

RESULTS AND DISCUSSION

The Table shows the results of two trials made with varying concentrations of phenol and the results are expressed as corrected % inhibition of germination rather than corrected % germination.

The conidial count technique is not a simple one in as much as it involves purification of *conidia*, a suitable nutrient medium, temperature control, time, and particularly, great patience. Conidia clump together and the germ tubes become tangled which makes counting difficult. Centrifugation encourages the clumping of conidia. The exploration of added surfactant would perhaps minimize this tendency to clump and perhaps conidial suspensions of some thousands or hundreds would be better than the concentrations used in this study. Temperatures between 28 and 30 C produced reasonably rapid germination in from 12 to 18 hours. On the other hand, temperatures between 23 and 25 C showed a marked slowing of germination. A conidial germination technique would appear feasible but it would be time-consuming.

REFERENCES

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